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Occurrence of *Giardia* and *Cryptosporidium* spp. in Surface Water Supplies

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***Giardia* and *Cryptosporidium* levels were determined by using a combined immunofluorescence test for source waters of 66 surface water treatment plants in 14 states and 1 Canadian province. The results showed that cysts and oocysts were widely dispersed in the aquatic environment. *Giardia* spp. were detected in 81% of the raw water samples. *Cryptosporidium* spp. were found in 87% of the raw water locations. Overall, *Giardia* or *Cryptosporidium* spp. were detected in 97% of the raw water samples. Higher cyst and oocyst densities were associated with source waters receiving industrial or sewage effluents. Significant correlations were found between *Giardia* and *Cryptosporidium* densities and raw water quality parameters such as turbidity and total and fecal coliform levels. Statistical modeling suggests that cyst and oocyst densities could be predicted on the basis of watershed and water quality characteristics. The occurrence of high levels of *Giardia* cysts in raw water samples may require water utilities to apply treatment beyond that outlined in the Surface Water Treatment Rule of the U.S. Environmental Protection Agency.**

To address the increasing problem of waterborne outbreaks of disease, the U.S. Environmental Protection Agency (EPA) finalized the Surface Water Treatment Rule on 29 June 1989 (24). The rule requires filtration and disinfection of all surface water supplies (criteria are also specified to determine if a system could avoid filtration technology), primarily as a means of controlling *Giardia* spp. and enteric viruses.

For systems that filter and disinfect, the rule stipulates that utilities would be required to meet design and operating criteria specified by the state (or other primacy agency) to ensure overall removal and/or inactivation of at least 99.9% of *Giardia* cysts and 99.99% of enteric viruses.

The intent of the Surface Water Treatment Rule was to reduce the risk of acquiring a waterborne infection of *Giardia* spp. to below an annual rate of 10^{-4} per person (24). The EPA has determined that one case of microbiologically caused illness per year per 10,000 people is a reasonable goal (24). To achieve a 10^{-4} annual risk of *Giardia* infection, it has been calculated that potable water should not contain more than 7×10^{-4} *Giardia* cysts per 100 liters on the basis of the geometric mean for 1 year (12). Therefore, the requirement for 99.9% removal by treatment plants assumes that *Giardia* levels in raw water are not greater than 7 cysts per 100 liters. Although the rule specifies that greater treatment may be required for water with poor water quality, no data are given regarding the distribution of *Giardia* spp. in various water supplies.

In recent years, *Cryptosporidium parvum* has been recognized as an agent of waterborne disease (3–6, 11, 19). The EPA has proposed to include *Cryptosporidium* spp. in the next series of regulations (25). Information on the occurrence and distribution of *Cryptosporidium* oocysts in raw water supplies will be necessary to evaluate the impact of any such regulation.

The purpose of this project was to examine 66 surface water filter plants for the occurrence and distribution of *Giardia* and *Cryptosporidium* organisms in the raw water supplies. The occurrence of these organisms was related to a variety of source water characteristics. Finally, the levels of cysts and oocysts in raw water supplies were evaluated within the context of the Surface Water Treatment Rule.

MATERIALS AND METHODS

Sample collection. The methods for simultaneous detection of *Giardia* and *Cryptosporidium* spp. have been previously presented (7, 8). Samples were collected from 66 surface water treatment plants in 14 states and 1 Canadian province (Fig. 1). Raw water samples were collected by using a gasoline-driven water pump (Arkos SA18 pump, Brescello, Italy) or a pressurized tap and filtered through 10-in. (25.4-cm) wound polypropylene cartridge filters having a nominal porosity of 1 μm (Filterite Corp., Timonium, Md.; catalog no. U1A10U). Flow rates were adjusted to 1 to 3 gal/min (1 gal = 3.785 liters) (measured by use of a Kent model C700 flowmeter placed downstream of the filter), and approximate volumes of 100 gal (378 liters) were collected. Between samples, the units were flushed with 30 gal (113 liters) of tap water to dislodge any attached organisms.

After collection, the filter along with the filter housing water was placed in a Whirl-Pac bag (Nasco, Fort Atkinson, Wis.) containing 10 ml of 37% formalin. The filters were double-bagged and shipped to the laboratory via overnight delivery. After delivery, the samples were stored at 2 to 5°C and processed within 24 to 72 h.

Sample clarification. To prepare samples for analysis, filters were cut in half lengthwise to the plastic core by using a sterile surgical scalpel to produce fibers approximately 2-in. (5.1-cm) long. Fibers were teased apart and placed in a 3,500-ml-capacity sterile stomacher bag (Tekmar Co., Cincinnati, Ohio) with 1.75 liters of phosphate-buffered saline (PBS; pH 7.4) containing 0.1% sodium dodecyl sulfate (SDS) and 0.1% Tween 80 (both from Sigma Chemical Co., St. Louis, Mo.). The filter material was homogenized (stomacher lab blender model 3500; Tekmar) for three 3-min intervals over a 15-min period. Between each homogenization period, the filter material was hand kneaded to redistribute the fibers in the bag. After homogenization, small

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FIG. 1. Location of sampling sites within the United States. In addition, one utility from Alberta, Canada, was examined (not shown). Because of the close proximity of some utilities, a single point may indicate more than one sample site.

portions of the filter material were hand wrung to remove the eluant water. Filter material was visually inspected before being discarded to ensure that no debris remained in the filter. Fibers containing debris were rewashed.

The homogenized samples were combined with the housing water and concentrated into a single pellet by centrifugation (1,050 × *g*, 10 min, 4°C) by using a 275-ml-capacity swinging-bucket rotor (model HS-4) and a Sorvall RC-5B refrigerated superspeed centrifuge (DuPont Co., Wilmington, Del.).

A 1-ml sample of the pellet was resuspended in 19 ml of eluant water and sonicated for 10 min. The sample was carefully underlayered with 30 ml of Percoll-sucrose (specific gravity, 1.10; Sigma) flotation medium and centrifuged at 1,050 × *g* for 10 min. The Percoll-sucrose gradient was prepared by following the instructions of the manufacturer (18) and stored at 2 to 5°C. The solution consisted of 52.6 ml of Percoll (specific gravity, 1.13), 10 ml of 2.5 M sucrose solution, and 37.4 ml of water. The specific gravity of the solution was checked by use of a hydrometer at 2 to 5°C. The Percoll-sucrose solution was maintained at 2 to 5°C throughout the experiment.

The top water layer (containing cysts) and 5 ml of the Percoll interface were drawn off, diluted to 50 ml by using elution water in a conical centrifuge tube, and recentrifuged at 1,050 × *g* for 10 min. The supernatant was siphoned off to a volume of 5 ml (plus pellet) and vortexed.

Labeling procedure. Sample concentrates along with positive controls (supplied by Meridian Diagnostics Inc., Cincinnati, Ohio) and sterility controls (i.e., elution water, Percoll-sucrose, etc.) were pipetted directly onto prewetted 25-mm cellulose acetate filters (0.2-μm pore size; Sartorius Inc., Hayward, Calif.) in the stainless-steel wells of a Hoefer manifold (Hoefer Scientific, San Francisco, Calif.). To ensure even distribution of sample, a 25-mm Durapore HVLP filter (0.45-μm pore size; Millipore Corp., Bedford, Mass.) was used under the cellulose acetate filter. Sample volumes, typically 0.1 to 0.5 ml, were used to provide a monolayer of material on the filter surface. The sides of the filter units were rinsed with SDS-Tween-PBS to ensure that all organisms were deposited on the filters. Vacuum was maintained at 3.0 to 5.0 in. Hg (ca. 76 to 127 mm Hg; 100 mm Hg = 133.322 Pa) by using a bleeder valve on the vacuum system.

Monoclonal antisera specific for *Giardia* and *Cryptosporidium* spp., developed by C. R. Sterling (20, 21) and distributed by Meridian Diagnostics (Hydrofluor-Combo kit), were diluted 1:10 with PBS. The combined antisera (0.5 ml) were pipetted and allowed to contact the entire filter for 25 min. Labeling reagent (0.5 ml of fluorescein-labeled anti-murine immunoglobulin), also part of the Hydrofluor-Combo kit, was added to the filters and allowed to react for 25 min. During the contact time, individual wells were covered with a no. 6 stopper to prevent dehydration and crystallization of the fluorescein isothiocyanate dye. Between and after the reagent additions, the filters were rinsed with PBS.

Microscopic examination. Filters were dehydrated with an alcohol series (10, 20, 40, and 80% ethanol–5% glycerol) and rinsed twice with a 95% ethanol–5% glycerol solution (16). Filters were placed on prewarmed microscope slides and cleared by using a 2% 1,4-diazabicyclo[2.2.2]octane (Aldrich Chemical Co., Milwaukee, Wis.)-in-glycerol solution. The coverslip was sealed by using clear nail polish and examined at 300× magnification by epifluorescent microscopy with an Olympus BH-2 research-grade microscope equipped with a 100-W high-pressure mercury lamp, a B-G dichroic mirror, an EY-455 exciter filter, and a PM-10ADS automatic photomicrographic system. The microscope was equipped with a modified BH2-NIC attachment (with phase annuli for 40× and 100× objectives) for phase-contrast and Nomarski differential interference contrast (DIC) microscopy.

Giardia spp. were identified by the cyst size (6 to 16 μm) fluorescence, and characteristic shape. Cysts were determined to have viable type morphologies (hyaline appearance and a peritrophic space) by use of the criteria of Schupp and Erlandsen (17) by DIC microscopy. It was found that fixing the cysts with 1 to 2% formalin preserved the morphological structures during sample processing.

Cryptosporidium spp. were determined by fluorescence, size (3 to 7 μm), shape, and surface texture (indicative of a sphere). The presence of sporozoites or a densely packed cytoplasm within the oocysts was observed by either phase-contrast or DIC microscopy. In some oocysts, densely packed cytoplasm made it difficult to distinguish the sporozoites. Because it was not possible to be certain that these organisms did not contain sporozoites, they were considered to be potentially viable.

Densities of parasites were reported in numbers of organisms per liter for surface water and backwash samples and numbers of organisms per 100 liters for tap water samples. When parasites were not detected, the parasite level was reported as less than the detection limit. Unless stated differently, values are not adjusted to reflect recovery efficiencies. Previous evaluations showed that the immunofluorescence procedure used had average recovery efficiencies of 48% for *Giardia* spp. and 42% for *Cryptosporidium* spp. in raw water samples containing 150 nephelometric turbidity units (8).

Water quality data. Additional water quality data (e.g., coliform counts, turbidity levels, disinfectant residuals, etc.) were provided by the participating utilities. All analyses were performed by state-certified laboratories and conducted according to accepted procedures.

Statistics. Statistical analyses were performed on logarithmically transformed data by use of the Statpack statistical package (Northwest Analytical, Portland, Oreg.).

RESULTS AND DISCUSSION

Distribution of cysts and oocysts in water. The results of this study found that *Giardia* and *Cryptosporidium* spp. were

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TABLE 1. Summary of *Giardia* and *Cryptosporidium* results

Date (day-mo-yr)	Site code	State or province	No. of organisms per liter: *Giardia* cysts	No. of organisms per liter: *Cryptosporidium* oocysts	Treatment goal[a]
14 Dec 88	102	N.J.	0.22	3.96	3.91
4 Jan 89	605	Ill.	5.87	74.85	5.34
18 Jan 89	604	Ill.	24.38	243.83	5.96
24 Jan 89	410	Pa.	13.73	135.36	5.71
31 Jan 89	410b	Pa.	1.32	8.98	4.69
7 Feb 89	504	Tenn.	16.51	19.81	5.79
14 Feb 89	703	Calif.	<0.51	3.05	
22 Feb 89	306	Pa.	0.04	0.07	3.20
27 Feb 89	506	Va.	17.37	10.86	5.81
7 Mar 89	314	Pa.	<0.79	2.36	
15 Mar 89	307	Pa.	30.12	<1.59	6.05
22 Mar 89	109a	Conn.	0.84	0.84	4.50
11 Apr 89	312a	Pa.	1.00	0.92	4.57
17 Apr 89	312b	Pa.	8.72	12.36	5.51
17 Apr 89	312c	Pa.	8.72	12.36	5.51
25 Apr 89	616	Ohio	0.96	14.42	4.55
1 May 89	512	W.Va.	1.90	4.76	4.85
9 May 89	101	N.J.	<1.80	3.61	
23 May 89	305	Pa.	7.03	2.55	5.42
31 May 89	609	Ohio	<1.76	3.52	
6 Jun 89	513	W.Va.	14.33	<3.58	5.73
13 Jun 89	310	Pa.	1.48	0.59	4.74
27 Jun 89	608	Ill.	<3.67	14.67	
27 Jun 89	615	Mo.	<241.98	484.27	
11 Jul 89	605	Ill.	6.07	59.19	5.35
18 Jul 89	514	W.Va.	0.37	0.55	4.14
25 Jul 89	311	Pa.	1.85	2.90	4.84
1 Aug 89	502	Ky.	<0.39	1.55	
14 Aug 89	401	Pa.	<0.53	1.06	
22 Aug 89	504	Tenn.	1.06	2.11	4.59
29 Aug 89	508	W.Va.	0.32	1.16	4.07
5 Sep 89	402	Pa.	0.22	1.54	3.91
11 Sep 89	503	Md.	3.24	1.08	5.08
19 Sep 89	618	Ohio	1.06	2.83	4.60
25 Sep 89	307	Pa.	1.70	0.85	4.80
10 Oct 89	511	W.Va.	0.88	<0.11	4.51
17 Oct 89	605	Ill.	8.34	41.70	5.49
24 Oct 89	405	Pa.	0.12	<0.12	1.66
31 Oct 89	610	Ind.	4.80	<1.20	5.25
21 Nov 89	516	W.Va.	4.01	1.18	5.17
27 Nov 89	611	Ind.	<0.28	0.85	
12 Dec 89	307	Pa.	10.58	1.92	5.59
13 Dec 89	603	Ill.	0.88	7.93	4.52
18 Dec 89	411	Pa.	1.78	0.20	4.82
19 Dec 89	619	Ohio	0.59	1.19	4.34
2 Jan 90	614	Mo.	2.91	1.19	5.03
3 Jan 90	501	W.Va.	66.04	66.04	6.39
9 Jan 90	302	Pa.	0.16	0.04	3.77
13 Jan 90	202	Ab.[b]	4.94	0.34	5.26
16 Jan 90	502	Ky.	<3.58	21.46	
16 Jan 90	502	Ky.	<1.54	1.54	
16 Jan 90	612	Ind.	7.82	1.42	5.46
23 Jan 90	315	Pa.	<0.22	<0.22	
23 Jan 90	414	Pa.	2.64	1.98	4.99
30 Jan 90	504	Tenn.	5.81	0.53	5.33
5 Feb 90	605	Ill.	<2.64	13.22	
5 Feb 90	605	Ill.	5.29	5.29	5.29
5 Feb 90	606	Ill.	5.29	5.29	5.29
19 Feb 90	605	Ill.	12.71	12.71	5.67
19 Feb 90	605	Ill.	2.71	10.83	5.00
19 Feb 90	606	Ill.	2.71	10.83	5.00
27 Feb 90	201	N.J.	17.16	1.52	5.80
27 Feb 90	307	Pa.	20.91	1.65	5.89
28 Feb 90	703	Calif.	<0.19	0.38	
20 Mar 90	109	Conn.	<0.48	<0.48	
20 Mar 90	509	W.Va.	1.06	0.53	4.59
27 Mar 90	404	Pa.	1.19	0.40	4.65
9 Apr 90	307	Pa.	17.17	0.66	5.80
9 Apr 90	307	Pa.	17.17	0.66	5.80
10 Apr 90	613	Ind.	12.38	6.19	5.66
23 Apr 90	307	Pa.	16.64	1.59	5.79
23 Apr 90	307	Pa.	16.64	1.59	5.79
1 May 90	519	W.Va.	5.28	<0.13	5.29
8 May 90	409	Pa.	30.21	<0.92	6.05
8 May 90	504	Tenn.	0.53	0.79	4.29
15 May 90	310	Pa.	0.60	0.36	4.35
15 May 90	602	Iowa	3.30	9.91	5.09
22 May 90	518	W.Va.	1.59	0.79	4.77
30 May 90	605	Ill.	14.80	14.80	5.74
5 Jun 90	406	Pa.	0.05	0.05	3.25
5 Jun 90	512	W.Va.	5.02	1.32	5.27
12 Jun 90	109	Conn.	<0.73	<0.73	
12 Jun 90	517	W.Va.	1.22	<0.15	4.66

[a] Treatment goal was calculated by adjusting the number of *Giardia* cysts per liter for the recovery efficiency and the average percent viability and determining the logarithmic difference between number of *Giardia* cysts per liter and 0.007 cyst per 100 liters. Calculations were not performed where no *Giardia* organisms were detected.

[b] Ab., Alberta, Canada.

widely distributed in the environment. *Giardia* spp. were detected in 69 of 85 (81.2%) raw water samples (Table 1). The geometric mean of (detectable) *Giardia* spp. was 2.77 cysts per liter, with levels ranging from 0.04 to 66 cysts per liter. These data are comparable to results from a number of investigators who found *Giardia* levels ranging from 0.006 to 6 cysts per liter (1, 9, 14, 15, 22). Reasons for the higher range of *Giardia* counts include differences in sample sites (most of the previous studies were conducted in relatively pristine waters) and recovery efficiencies due to different methodologies. Boutros (2) detected presumptive cysts in 38% of 50 surface water supplies in Pennsylvania which had previously been shown to be *Giardia* positive.

Cryptosporidium spp. were found in 74 of 85 raw water locations (87%), with levels ranging from 0.07 to 484 oocysts per liter (Table 1). The geometric mean of (detectable) *Cryptosporidium* levels was 2.70 oocysts per liter. The *Cryptosporidium* levels found in this study were comparable to the results of other investigators. Rose (11, 14) reported that 71% of 107 samples from the western United States contained *Cryptosporidium* oocysts. Geometric means of *Cryptosporidium* levels ranged between 0.91 and 28 oocysts per liter (14). Ongerth and Stibbs (10) estimated that *Cryptosporidium* levels in several western Washington and California rivers ranged between 2 and 112 oocysts per liter. Oocysts were detected in all of the river water samples examined. Boutros (2) detected presumptive *Cryptosporidium* spp. in 70% of the 50 surface water supplies in Pennsylvania. *Cryptosporidium* densities ranged from 0.002 to 4.49 oocysts per liter.

Overall, *Giardia* or *Cryptosporidium* spp. or both were detected in 97% of the surface water supplies. *Cryptosporidium* spp. averaged 1.5 times more numerous than *Giardia* spp. (Fig. 2). There was a significant correlation ($r = 0.59$, P

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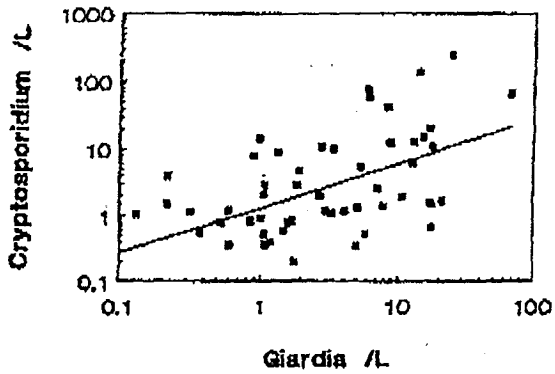

FIG. 2. Relationship between densities of *Cryptosporidium* oocysts and *Giardia* cysts in surface water. Regression line: $y = 0.667(x) + 0.104$; $r = 0.585$, $P < 0.01$.

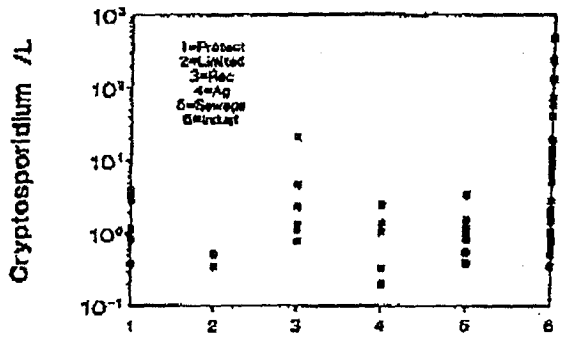

FIG. 4. Relationship between *Cryptosporidium* oocyst densities and source water protection. The key is the same as shown in the legend to Fig. 3.

< 0.01) between cyst occurrence and oocyst occurrence. These results are consistent with the findings of other researchers who reported that *Cryptosporidium* spp. are generally found in higher densities than *Giardia* spp. (13, 15). Rose et al. (13) found a significant correlation ($r = 0.778$, $P < 0.01$, $n = 39$) between *Cryptosporidium* and *Giardia* occurrences within a single watershed.

Estimate of viability. Because *Giardia* and *Cryptosporidium* spp. were frequently detected in surface water samples, it was important to determine whether the organisms were viable. Unfortunately, there are no reliable methods to determine the viability of individual cysts or oocysts observed in environmental samples. Viable type cysts (cysts with a hyaline appearance and a peritrophic space) were determined by using DIC microscopy by the procedures of Schupp and Erlandsen (17). *Cryptosporidium* spp. with sporozoites observed by either phase-contrast or DIC microscopy were considered potentially viable. The determination of viable type morphologies by using microscopic methods is broad and likely overestimates cyst and oocyst viability. However, the method is best thought of in terms of determining cell mortality. A viable type morphology does not imply that the organism can excyst or infect animals; rather, a cyst that does not have a viable type morphology, i.e., one that has a distorted or shrunken cytoplasm, is probably dead. Because water treatment practices are designed to kill pathogenic organisms, cysts and oocysts found in potable water supplies should clearly be nonviable. Control experiments showed cysts lost viable type morphologies when exposed to a free chlorine residual (data not shown).

Observation of 618 *Giardia* spp. in raw samples showed that viable type morphologies were seen in 12.8% of the samples. Approximately 32% of the 242 *Cryptosporidium* spp. observed in raw water samples contained sporozoites within the oocyst. These results suggest that the majority of cysts and oocysts observed in the samples were not viable.

Relationship between parasites and source type. Plant operators were asked to evaluate the degree of watershed protection for the source waters. Higher cyst and oocyst densities were associated with source waters receiving industrial or sewage effluents. Data shown in Fig. 3 indicate a progressive increase in *Giardia* levels in waters with decreasing watershed protection. On average, water receiving industrial (urban) pollution contained 10 times more *Giardia* organisms than protected watersheds. For purposes of tabulation, if a watershed received pollution from multiple sources, it was given the highest rating (i.e., water receiving agricultural, sewage, and industrial effluents was tabulated as industrial).

A less-clear relationship between source water protection and oocyst levels existed for *Cryptosporidium* spp. Figure 4 shows no significant difference in oocyst levels between protected watersheds and those receiving sewage treatment

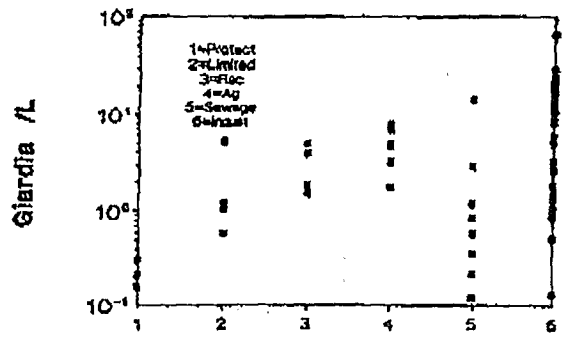

FIG. 3. Relationship between *Giardia* cyst densities and source water protection. Key: 1, protected watersheds; 2, watersheds with limited access; 3, recreational use; 4, agricultural use; 5, sewage discharge; 6, industrial-urban discharges.

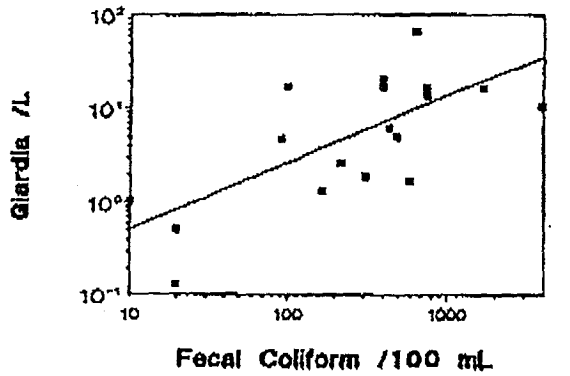

FIG. 5. Relationship between fecal coliform count and *Giardia* cyst densities. Regression line: $y = 0.711(x) - 0.997$; $r = 0.702$, $P < 0.01$.

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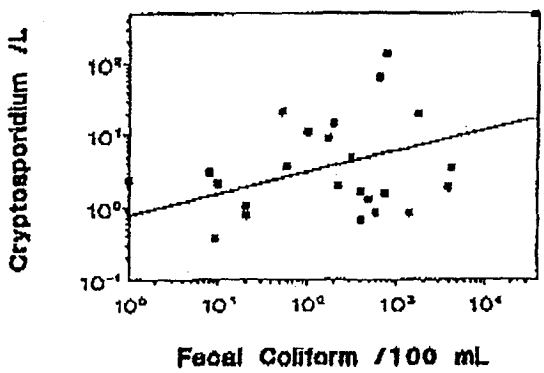

FIG. 6. Relationship between fecal coliform count and *Cryptosporidium* oocyst densities. Regression line: $y = 0.292(x) - 1.03$; $r = 0.383$, $P < 0.05$.

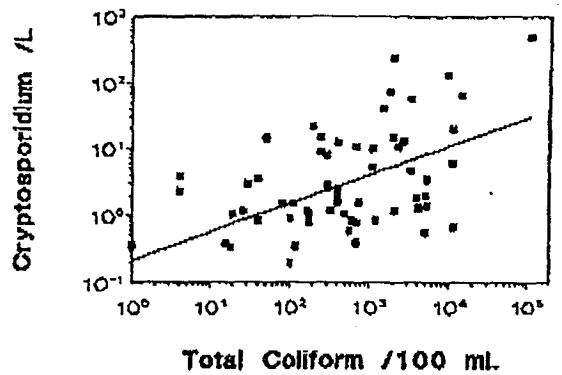

FIG. 8. Relationship between total coliform count and *Cryptosporidium* oocyst densities. Regression line: $y = 0.428(x) + 0.675$; $r = 0.541$, $P < 0.01$.

plant effluents. Oocyst levels associated with sites receiving industrial (urban) sources of pollution were approximately 10 times higher than in protected sites. Rose (13) reported *Cryptosporidium* levels 10 to 50 times higher in waters receiving sewage or agricultural pollution than in pristine waters.

There is a growing consensus among researchers that elevated *Giardia* levels are due to introduction of sewage effluents, while elevated *Cryptosporidium* levels may be due to input from nonpoint sources (23). The current research is consistent with this hypothesis. The implication of these results is important for the water industry as it raises concerns about the adequacy of disinfection practices at sewage treatment plants. Of particular concern is the practice of seasonal disinfection, where inactivation of organisms in sewage effluents is discontinued during winter months when cold water conditions favor cyst survival in treatment processes. While watershed control of point source pollution may aid in reducing *Giardia* levels, it is unknown whether these practices will be reliable for controlling *Cryptosporidium* levels. Additional research is necessary to evaluate the impact of watershed management programs on *Cryptosporidium* occurrence.

Consistent with the findings shown in Fig. 3 and 4 was the observation that the highest *Giardia* levels were detected in rivers and creeks. In many cases, these rivers and creeks also received sewage and industrial effluents. The highest parasite densities for both *Giardia* and *Cryptosporidium* spp. were found in midwestern rivers (i.e., Mississippi, Ohio, Missouri). *Cryptosporidium* levels in these major rivers ranged from 10 to 484 oocysts per liter.

Relationship between parasites and water quality. Data presented in Fig. 5 to 10 show positive relationships between *Giardia* and *Cryptosporidium* densities and raw water quality parameters such as turbidity, total coliform levels, and fecal coliform densities. A significant relationship was observed between *Giardia* densities and levels of fecal coliforms ($r = 0.70$; Fig. 5) and total coliforms ($r = 0.66$; Fig. 7). For both relationships, *Giardia* densities increased approximately 1 $\log_{10}$ for every 1.6 $\log_{10}$ increase in coliform levels. *Cryptosporidium* densities showed a significant correlation ($r = 0.75$) to raw water turbidity (Fig. 10).

These results are somewhat different from those found by Akin and Jakubowski (1) and Rose et al. (13). These investigators found no correlation between densities of *Giardia* or *Cryptosporidium* spp. and total or fecal coliform counts or turbidity. One reason for this discrepancy may be due to differences in the type of water samples analyzed. Most of the previous studies have examined relatively pristine waters, while the current study examined a wide variety of source waters. For example, Rose et al. (13), in their study, reported maximum total and fecal coliform counts of 286 and

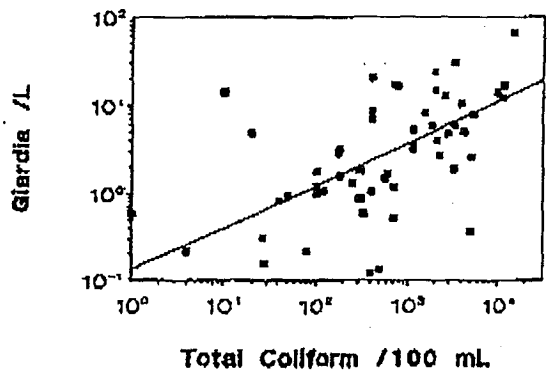

FIG. 7. Relationship between total coliform count and *Giardia* cyst densities. Regression line: $y = 0.483(x) - 879$; $r = 0.656$, $P < 0.01$.

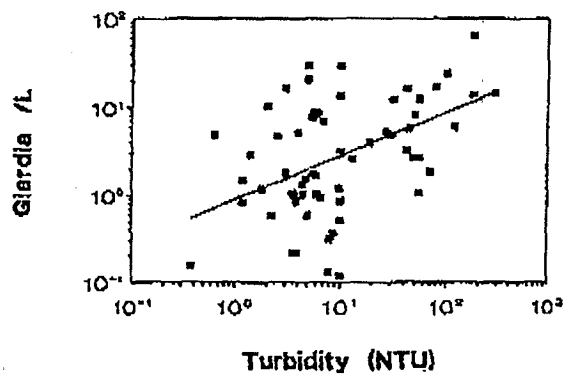

FIG. 9. Relationship between turbidity and *Giardia* cyst densities. Regression line: $y = 0.497(x) - 0.045$; $r = 0.443$, $P < 0.01$.

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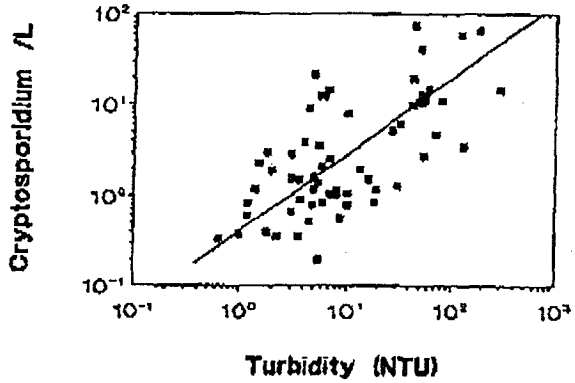

FIG. 10. Relationship between turbidity and *Cryptosporidium* oocyst densities. Regression line: $y = 0.847(x) - 392$; $r = 0.748$, $P < 0.01$.

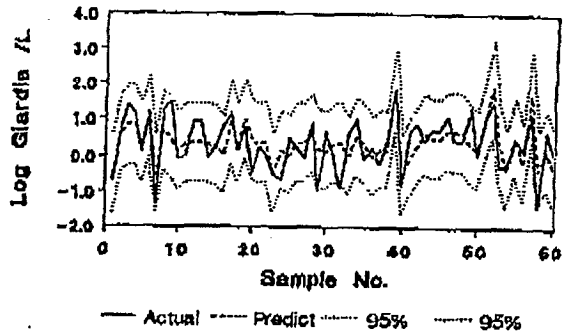

FIG. 11. Comparison of actual *Giardia* cyst data and the multiple linear regression model.

57 bacteria per 100 ml, respectively. In this study, maximum total and fecal coliform counts were 1×10^5 and 3×10^4 bacteria per 100 ml, respectively (Fig. 6 and 8). By examining all of the studies, it can be concluded that increasing levels of pollution (signaled by high counts of total and fecal coliform bacteria and high turbidities) represent an increased probability that *Giardia* and *Cryptosporidium* organisms will be present at elevated densities.

Statistical models. It was of interest to combine all of the raw water parameters into a model which could be used to predict *Giardia* and *Cryptosporidium* levels in water supplies. If such a model could be developed, it would be useful for predicting the level of treatment required for a particular water supply. Results shown in Table 2 indicate that 49.1% of the variation in raw water *Giardia* levels could be predicted by the level of watershed protection, raw water total coliform count, and turbidity. Figure 11 demonstrates the fit of the actual data to the predictive model. In certain cases the model exactly predicted peak *Giardia* levels. A similar model was developed for *Cryptosporidium* spp. (Table 3; Fig. 12). It shows that 51.9% of the variation in *Cryptosporidium* levels could be accounted for by the level of watershed protection, the type of water supply (e.g., river, lake, reservoir, etc.), raw water total coliform count, water temperature, pH, and average monthly turbidity. Both models were statistically significant ($P < 0.01$) and showed that parasite densities can be correlated to water quality parameters.

One of the most important parameters in each model was the level of watershed protection. This variable was assigned a numerical value according to a description provided by the water utility. In some cases, the judgment was purely subjective. For example, several utilities along the same river gave differing assessments of the level of watershed protection (these assessments were reviewed and corrected). However, future experiments should measure more objective assessments of sewage contamination (fecal coliform, ammonia, nitrate, phosphate, chlorophyll *a*, etc.). In this study, fecal coliform data were collected (Fig. 7 and 8), but data were available from only one-fourth of the water utilities.

Evaluation of treatment goals. Rose (12) estimated that a 1/10,000 annual risk of *Giardia* infection would result from an exposure to an annual geometric mean of 0.0007 cysts per 100 liters. Depending on the level of contamination in the raw water supply, utilities will have to apply treatment to achieve a geometric mean of <0.0007 cyst per 100 liters in finished water. Insufficient data prevents the development of a similar risk model for *Cryptosporidium* spp.

To calculate the amount of treatment required to reach the *Giardia* risk assessment goal, raw water values were adjusted for the method recovery efficiency (48%) and for cyst viability (13%). Overall, it is estimated that the average utility will have to apply 5.0 $\log_{10}$ removal and/or inactivation to achieve an annual risk of *Giardia* infection of <1/10,000 (Table 1). In only the most pristine situations, where raw water counts were <0.07 cyst per liter, would a utility require only a 3 $\log_{10}$ removal of *Giardia* spp.

Moreover, utilities should treat for the peak *Giardia* occurrence in the watershed. A spike of *Giardia* cysts after

TABLE 2. Multiple linear regression model for *Giardia* spp.[a]

Parameter[b]	Coefficient	SE	*t* statistic	Standard coefficient	Contr. r^2
Log *Giardia* spp. =					
Intercept	−0.6934	0.2239	−3.097		
Protect	0.1682	0.0450	3.735	0.371	0.127
Coli	5.14e-5	1.50e-5	3.427	0.337	0.107
NTU	0.0044	0.0014	3.214	0.316	0.094

[a] The corrected r^2 for 0.491 was 0.464; the F test was 16.038.
[b] Protect, code for the level of watershed protection; Coli, total coliform; NTU, turbidity (nephelometric turbity units). Total degrees of freedom (df) is 59.

TABLE 3. Multiple linear regression model for *Cryptosporidium* spp.[a]

Parameter[b]	Coefficient	SE	*t* statistic	Standard coefficient	Contr. r^2
Log *Cryptosporidium* spp. =					
Intercept	−1.6704	1.2253	−1.363		
Type	−0.2164	0.1022	−2.117	−0.216	0.038
Protect	0.1275	0.0570	2.237	0.266	0.042
Coli	1.87e-5	5.79e-6	3.226	0.309	0.088
Temp	−0.0131	0.0092	−1.422	−0.140	0.017
pH	0.2742	0.1673	1.638	0.182	0.023
MNTU	0.0032	0.0016	1.949	0.199	0.032

[a] The corrected r^2 for 0.519 was 0.468; the F test was 10.237.
[b] Type, code for water type (i.e., river, lake, etc.); Protect, code for the level of watershed protection; Coli, total coliforms; Temp, water temperature; MNTU, average monthly turbidity (nephelometric turbity units). Total degree of freedom (df) is 63.

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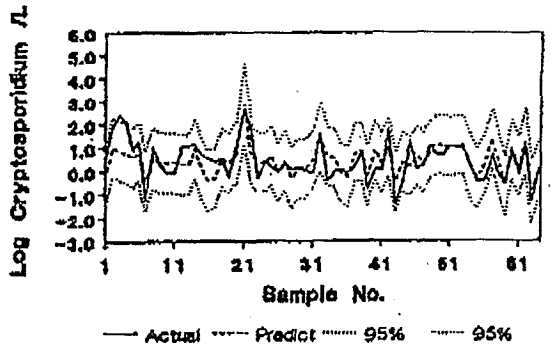

FIG. 12. Comparison of actual *Cryptosporidium* oocyst data and the multiple linear regression model.

a rainstorm event or a breakdown in upstream sewage treatment could overwhelm potable water barriers and result in waterborne disease. Protection of watersheds used for potable water, proper handling and continuous treatment of sewage effluents, and vigilant monitoring are required to control the occurrence of cysts and oocysts in source waters. Clearly there is a need for utilities to perform evaluations of raw water parasite levels to determine the appropriate level of treatment.

Additional research is needed to develop reliable methods for determining cyst and oocyst viability. Studies should also be performed to evaluate the virulence of environmental cysts and oocysts. Such information is critical to determine the significance of, and develop appropriate regulation for, waterborne parasites.

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